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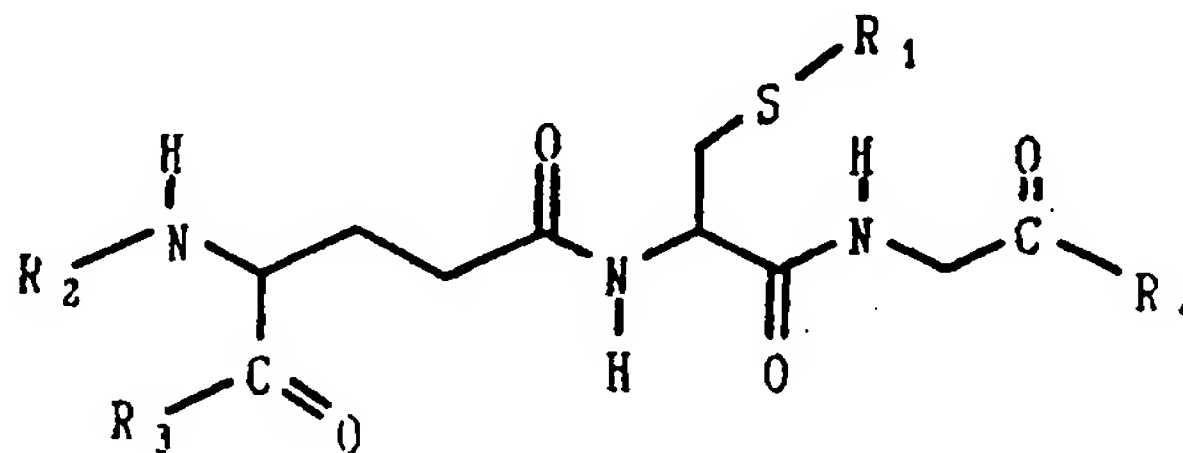
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(54) Title: S-BLOCKED GLUTATHIONES



(57) Abstract

This invention concerns glutathione derivatives, and in particular their application in the suppression of pathogens. It has been discovered that certain glutathione derivatives are effective inhibitors of the growth of a range of cancer cell types, and certain micro-organisms. According to one aspect of the present invention there is provided a glutathione having structure (I). Compounds based upon this general structure are disclosed which are active against parasitic infectious agents such as T. Brucei and L. Donovanii. Further compounds are disclosed which are active against cancer cells.

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S-Blocked Glutathiones

This invention concerns glutathione derivatives, and
5 in particular their application in the suppression of
pathogens.

Illness may be caused by many agents. Bacterial
infections are caused by micro-organisms which
10 multiply rapidly and cause a number of diseases.
Parasites are organisms which live in or on a host and
feed off the host. Cancers are evident by the
uncontrolled multiplication of cells in the body. The
cancer may be localized, such as breast cancer, or
15 systemic such as leukaemia.

The treatment of the illnesses caused by the
aforementioned agents has been the subject of much
research, and many different approaches. One approach
20 involves targeting the cells which cause the disease
and destroying them or disrupting their ability to
multiply.

For such an approach to be successful the agent or
25 drug used to attack the diseased cells should not harm
significantly other healthy cells. Thus such an

approach requires an understanding and identification of the biochemical processes carried out in cells, and the targeting and disruption of specific processes which are unique to the diseased cells.

5

Such an approach involves the use of the cytotoxic compound methylglyoxal which is produced in the cells of certain organisms. A build up of methylglyoxal in the cell up to cytotoxic levels will, of course, result in cell death. By inducing a build up of methylglyoxal, significant growth inhibition effects have been seen in tumour cells, *Escherichia coli*, *Saccharomyces cerevisiae* and *Leishmania donovani*.

10 The build up of methylglyoxal may be promoted by the inhibition of glyoxalase I (GLI) enzyme. A wide range of inhibitors of GLI are known and these include substrate or product analogues and mechanism-based inhibitors.

20

Glutathione (γ -glutamylcysteinylglycine) fulfils a variety of roles vital to life processes. It functions as a co-enzyme, co-substrate, substrate or part of the substrate architecture. S-blocked glutathiones have been shown to be potent inhibitors of GLI in vitro and this has led to a search for particular S-blocked

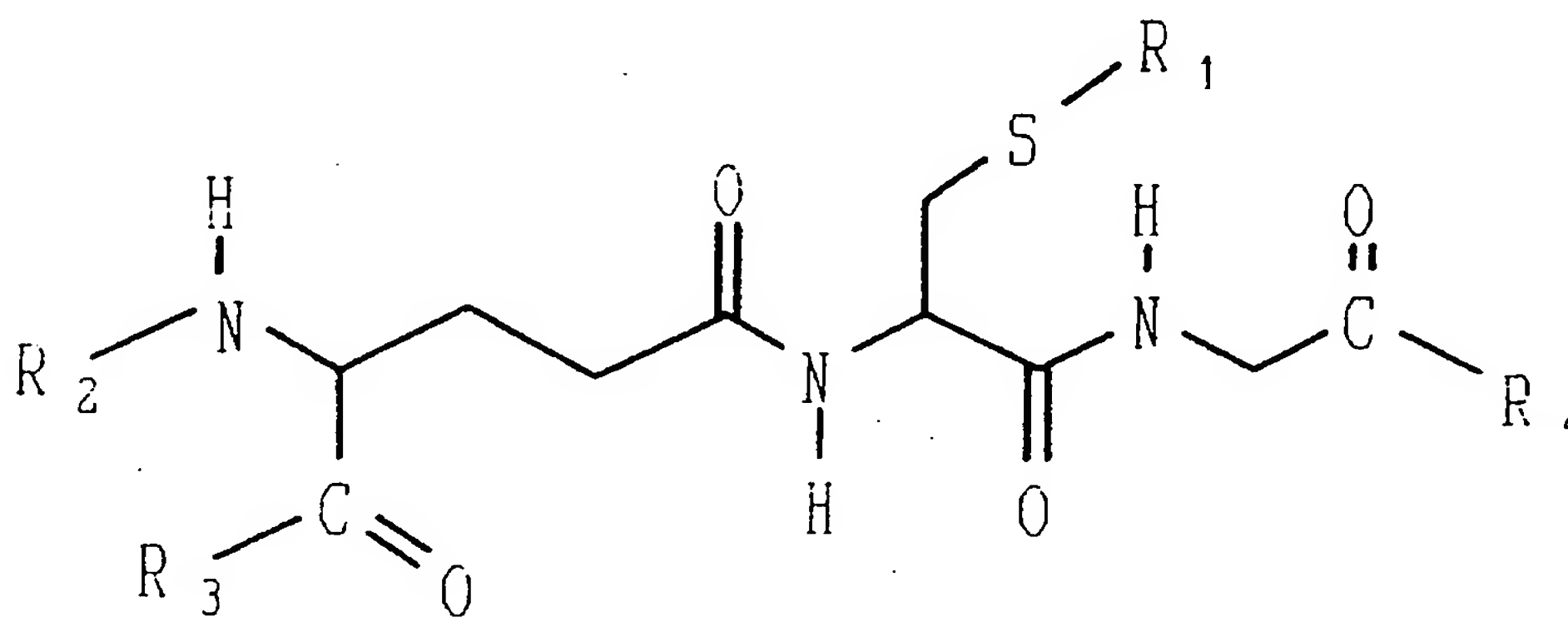
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glutathiones which may have therapeutic effect.

A general procedure for the preparation of the monoglycyl and dimethyl ester and amide derivatives of
5 S-(4-bromobenzyl)glutathione has been described by the inventor in Biochem. J. (1990) 271, pp167-169.

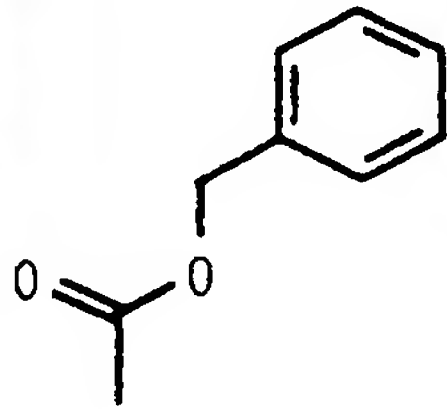
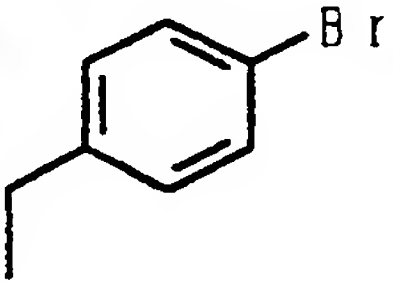
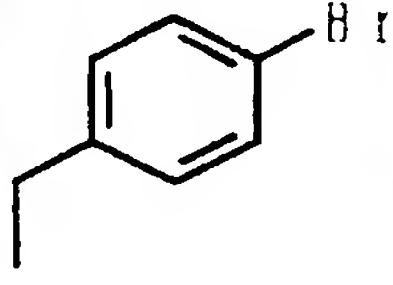
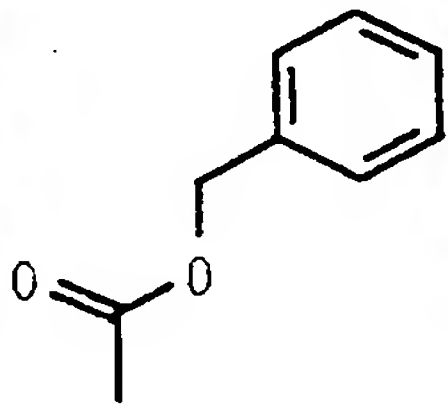
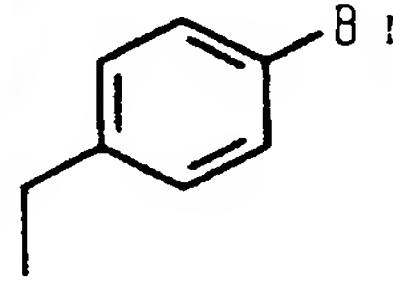
The inventor has discovered that certain glutathione derivatives are effective inhibitors of the growth of
10 a range of cancer cell types, and certain micro-organisms.

According to one aspect of the present invention there is provided a glutathione having the following
15 structure:-

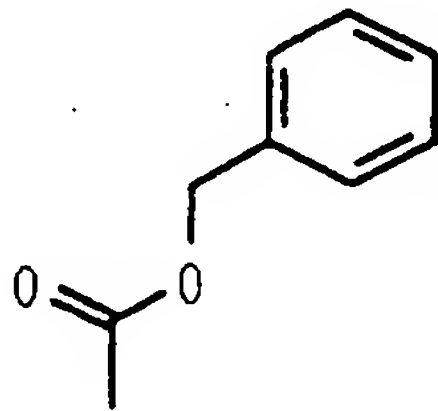
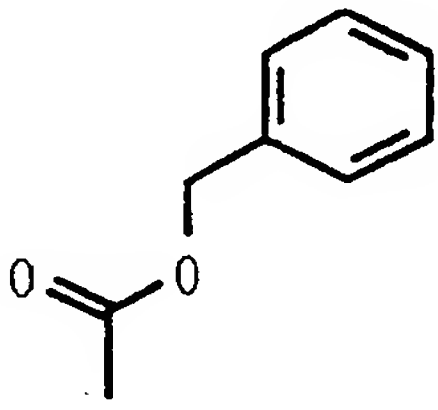
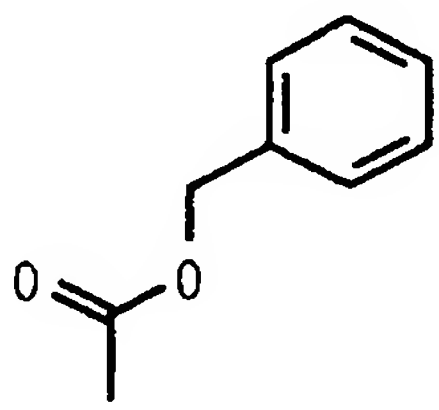
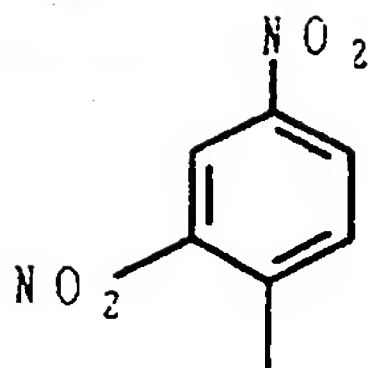


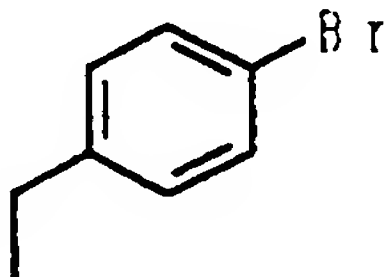
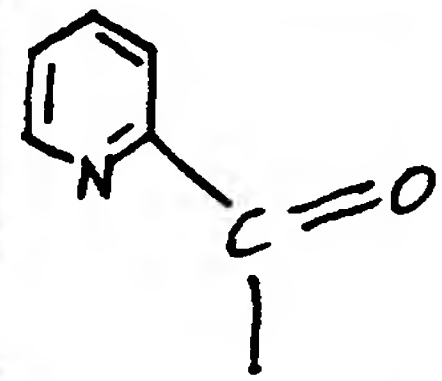
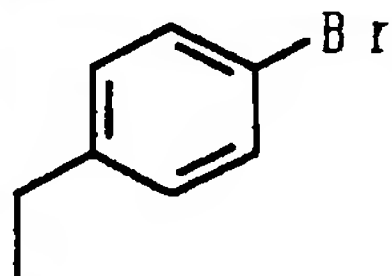
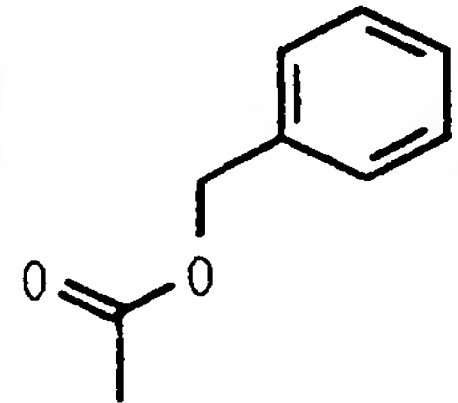
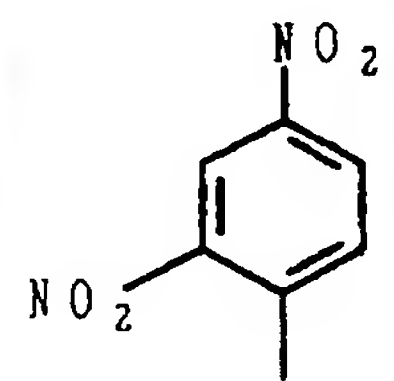
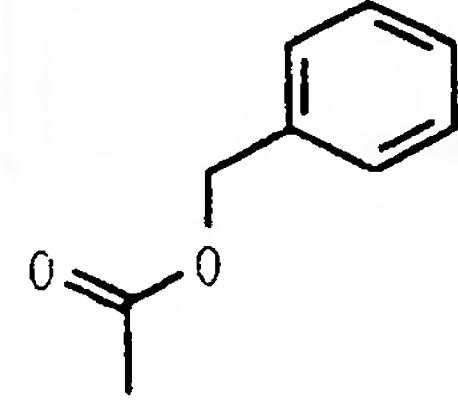
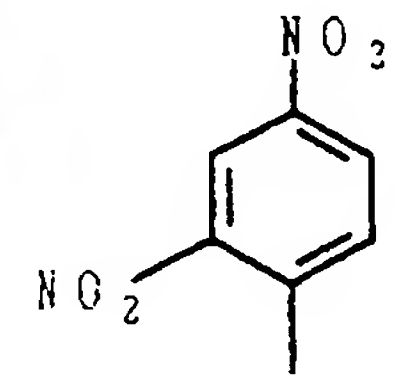
Where R₁-R₄ may be configured as shown in the following:

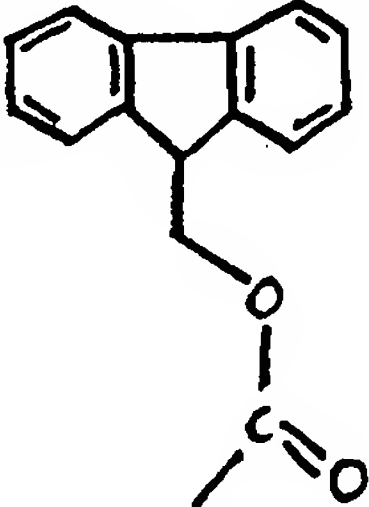
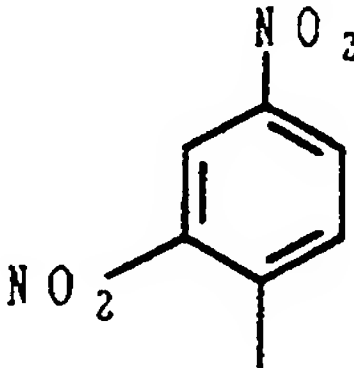
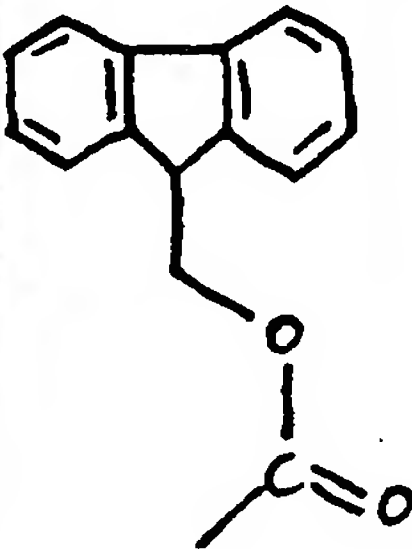
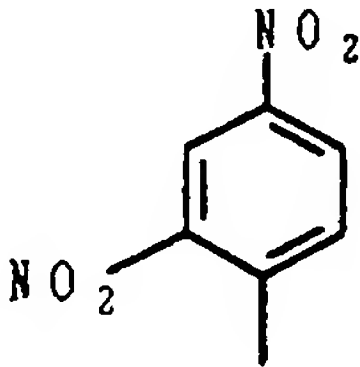
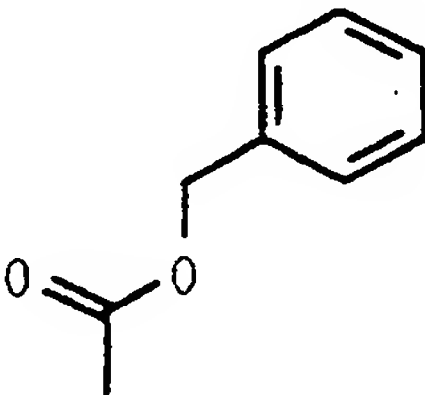
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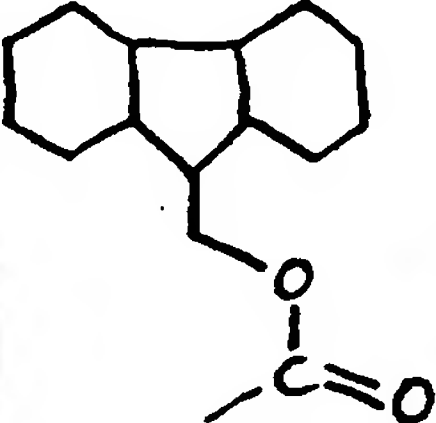
LABEL	R ₂	R ₁	R ₄	R ₃	MW
CD4			OMe	OMe	638.5
CD6	CH ₃ CO		OH	OH	545
CD7			OMe	OH	624

5

CD8		CH ₂ -COOEt	OH	OH	527
CD10			OMe	OMe	692
LABEL	R ₂	R ₁	R ₄	R ₃	MW
CD13	H		OH	OH	-

CD16	CHO		OH	OH	504
CD17			NH ₂	OH	576
CD19			OMe	OH	621
CD20			OMe	OMe	635

CD42			OMe	OH	708
CD43			OMe	OMe	722
CD46		-CH ₂ -CO ₂ Et	OMe	OMe	555

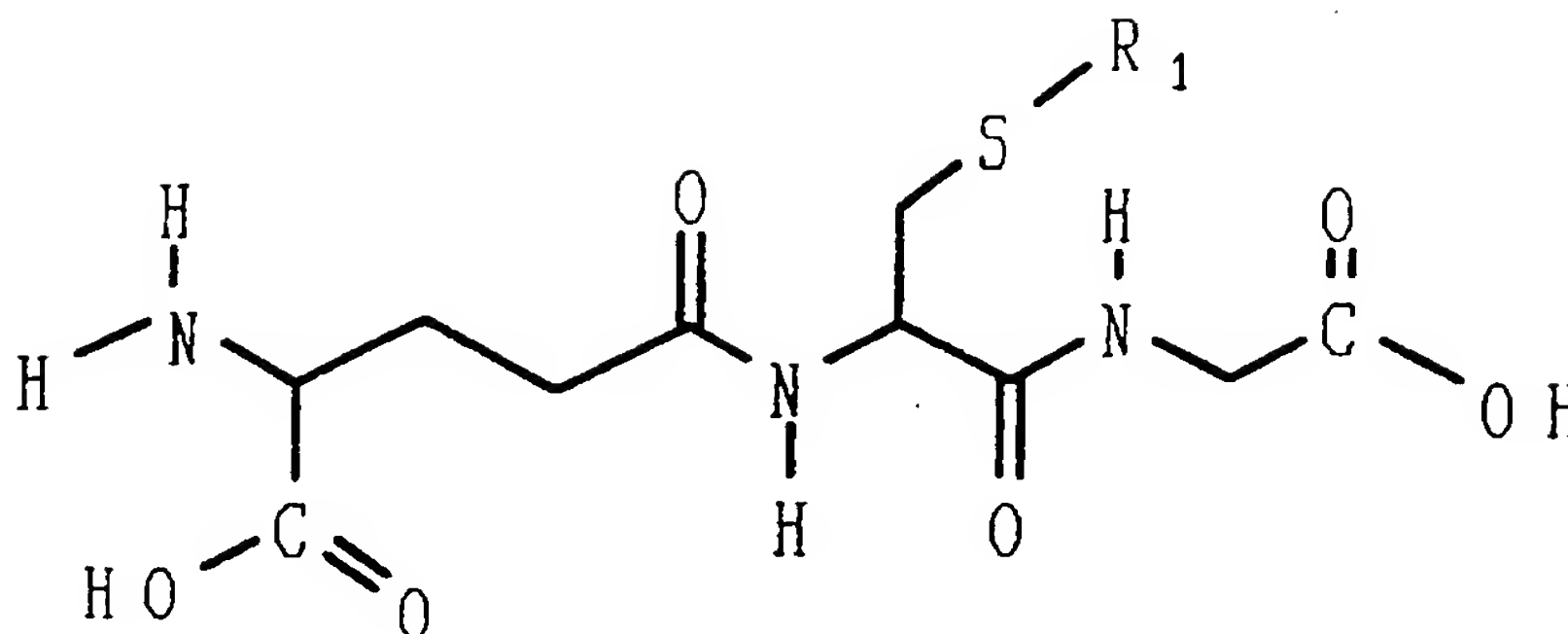
CD48	CH ₃ CO		OMe	OH	556
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CD7 has been found to be an effective inhibitor of Trypanosomiasis and in particular T. Brucei S247. This compound is also active against Malaria. One particular advantage of this compound is that it is effective at inhibiting the cell growth of the organism without being toxic to red blood cells.

CD13 is effective at the inhibition of growth of cancer cells, and in particular leukaemia, breast cancer or tumour cells.

According to another aspect of the invention there is provided a glutathione having the following general formula:-

5 Wherein R₁
is:-



10 CH₂CH=C(
CH₃)CH₂CH₂CH=C(CH₃)CH₂CH₂CH=C(CH₃)₂
TRANS TRANS TRANS
S-(farnesyl)glutathione CD37

15 The aforementioned glutathione derivatives may each be
provided in pharmaceutically acceptable compositions
for delivery to the human or animal body.

20 In another aspect of the invention there is provided
the use of any one of the foregoing compounds in the
treatment of cancer, or parasitic cellular
infestation.

25 In particular, according to one embodiment of the
present invention there is provided the use of CD13
and derivatives thereof in the treatment of cancer.

According to another embodiment there is provided the

10

use of CD19 and/or CD20 in the treatment of a
infection by a parasitic micro-organism.

In particular Cd19 is highly effective at low
5 concentrations against T. brucei (African sleeping
sickness), while CD 20 has no toxicity to red blood
cells. CD20 also has good activity against other
parasites such as L.donovani (oriental sore, Kala-
azar).

10

According to another aspect of the invention there is
provided the use of CD7 against malaria.

According to yet another aspect of the invention there
15 is provided the use of any one of compounds CD26-CD37
against cancer.

According to another aspect of the invention there is
provided the use of CD13, CD4, CD6 and CD 37 against
20 cancer, and in particular breast cancer, and more
particularly MCF7 cells.

Following is a description by way of example only of
methods of putting the present invention into effect
25 and examples demonstrating the activity of compounds
according to the present invnetion. The drawing is a

11

graphic representation of the results of the in vivo test described in example 1.

General method of production.

5



Reduced glutathione (1g, 3.26mM) is dissolved in H₂O (5ml) and 2M NaOH (3.3ml, 6.6mM) with stirring at room temperature and under a nitrogen atmosphere. Ethanol (5-15ml) is then added to the cloud point whereafter RX (for example, aryl halide, 3.5mM dissolved in ethanol) is added portion-wise over about 30 minutes. The reaction is left to stir for 20 hours under nitrogen.

If precipitation occurs during addition either more ethanol or more water is added to dissolve the material. At the end the reaction the acidity of the mixture is adjusted to p 3.5 with 2M HCl and the mixture chilled to effect precipitation. The precipitate is then filtered, washed with water, dried and recrystallized from Ethanol/H₂O.

25 Pharmaceutical activity:

Example 1

12

Glutathione CD13 according to the present invention was tested in order to ascertain its inhibitory characteristics with respect to various cancer cell lines.

5

The compound was introduced to cell cultures and the concentration of cancer cells formed over a period of time was measured using standard techniques. The following table indicates the results for CD13 and another glutathione derivative "control" by way of comparison:-

10

15

	Leukaemia lines		Tumour cells	Breast Cancer cells
	WEH1 3B	K562	MAC 15A	MCF7
Compound	conc. $\mu\text{g/ml}$	conc. $\mu\text{g/ml}$	conc. $\mu\text{g/ml}$	conc. $\mu\text{g/ml}$
Control	36	54	>100	>100
CD13	3.4	2.5	0.43	0.48

CD13 was tested in vivo on rodents with MAC 15A S/C Tumours by giving them a 20 mg/Kg daily dose for five days. The treatment resulted in a reduction in tumour size and a 45% reduction in tumour volume after 4 days. The effect of CD 13 in the above test is shown

13

in the graph of the drawings.

Example 2

- 5 The glutathione derivatives CD7 and CD 10 according to the present invention were tested for their activity against the parasites T.Brucei S247, L.donovani and T. cruzi.
- 10 The following table shows the mean estimation of growth of T.Brucei S247 in a 72 hour incubation in the presence of a control glutathione derivative and CD10 in various concentrations:-

15

Compound	MIC @ concentration (μ M)			
	30	10	3	1
control	++++	++++	+++++	+++++
CD7	0	++++	+++++	+++++
CD10	0	++++	+++++	+++++

20

The forgoing table shows that Compounds CD7 and CD10 exhibit complete inhibition of the growth of T.Brucei S247 over the specified period at concentrations of 30 μ M.

25

14

The following table shows the results of the inhibition of the growth of *L. donovani* and *T. Cruzi* by compounds CD7, CD10 according to the present invention and "control" by way of comparison, at various concentrations.

Compound	% Inhibition of <u>L.donovani</u> @ conc.			% Inhibition of <u>T. Cruzi</u> @ conc.		
	90 (μ M)	30 (μ M)	10 (μ M)	90 (μ M)	30 (μ M)	10 (μ M)
control	0	0	0	0	0	0
CD7	0	0	0	0	0	0
CD10	T	T	0	T	T	T/0

10

The foregoing shows that compound CD10 shows good activity in the inhibition of growth of both *L. donovani* and *T. cruzi*.

15

Example 3:

20 Activity of compounds CD16-CD20 in Vitro.

Compound	% inhibition @ concn. (μ M)

15

5

	30	10	3	1
CD16				
T.cruzi	11.0	0	0	0
CD17				
T.cruzi	7.0	4.0	0	0
CD19				
T.brucei	100	100	33.2	0
CD20				
L.donovani	13.5	1.9	0	0
T.brucei	100	64.5	0	0

10

Example 4:

Compound CD48 was tested for its activity against cancer, with the following results for a range of cancer types:

15

Cancer	Human Ovarian carcinoma	Human lung carcinoma	Human colon carcinoma	Human myclogenious leukaemia	Mouse lymphoid neoplasm
Designa tion	A2780	H-460	BE	K562	P388

16

	μM	μM	μM	μM	μM
CD48	>50	>50	27.1	16.7	31.7

Example 5:

5

Compounds CD4, CD6, and CD 37 have also been found to be effective against various cancers, including breast cancer.

10 Example 6:

Compounds CD42 to 48 were tested for their activity against various parasitic infection agents and the results are shown in the following table:

15

Comp ound	% INHIBITION OF T.BRUCEI S247				%INHIBITION OF L. DONOVANI			
	30 μM	10 μM	3 μM	1 μM	30 μM	10 μM	3 μM	1 μM
CD42	100	100	0	0	1.2	0	0	0
CD43	100	100	0	0	T/O	0	0	0
CD44	100	100	100	63.2	T/ 100	0	0	0

20

17

CD46	0	0	0	0	66.2	0	0	0
					3			

Key: T/0 = toxic to macrophages / parasites present.

T/100 = toxic to macrophages / no parasites
present.

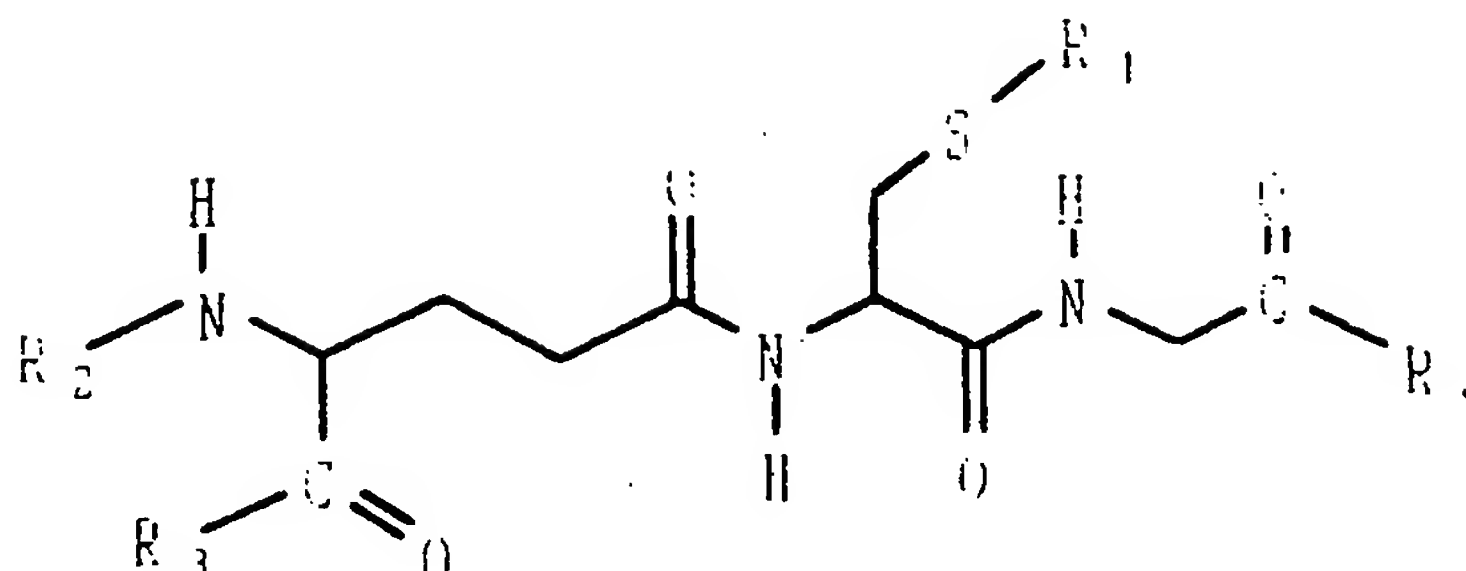
100+ = 100% inhibition when sampling for
haemocytometer count but parasites visible in
wells of 96-well plate under inverted mic.

10

18

CLAIMS

1. A compound having the following general structure:-

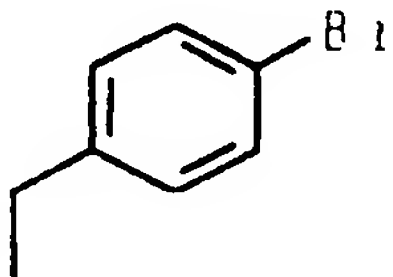
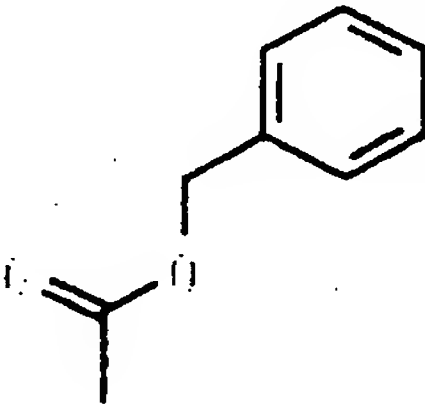
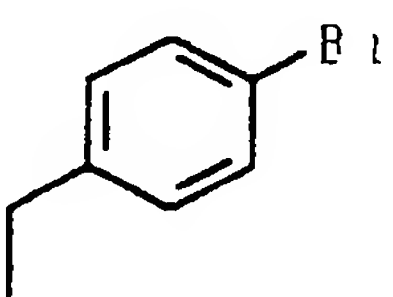
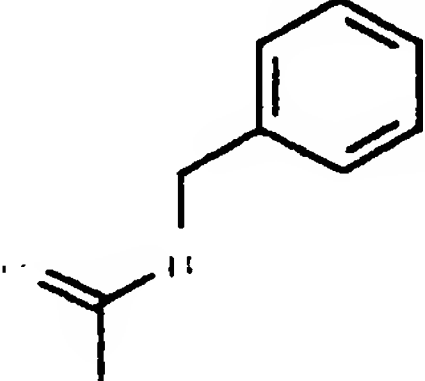


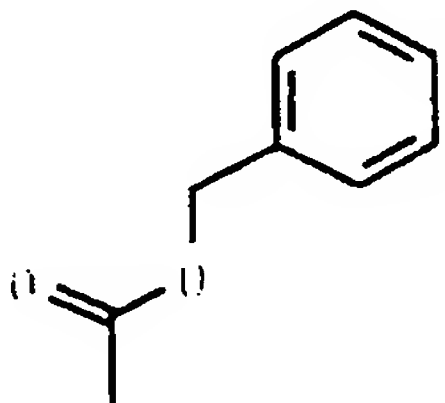
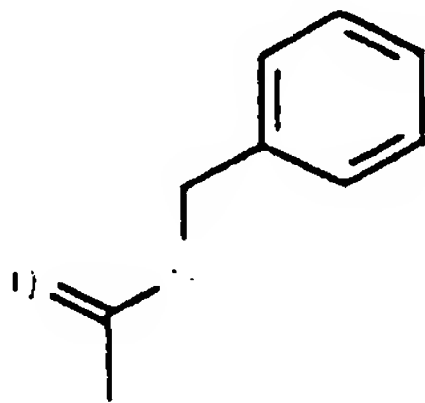
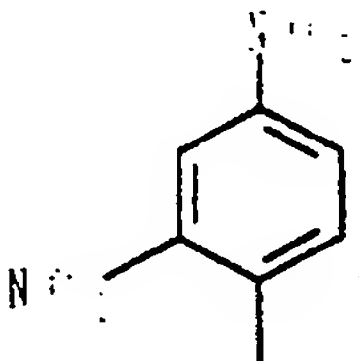
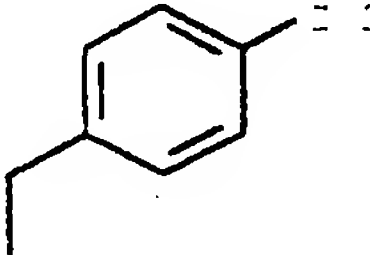
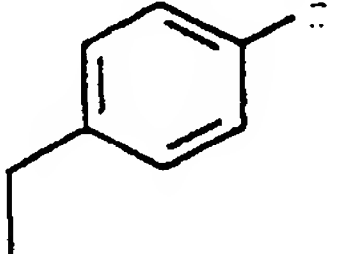
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and wherein R_1 to R_4 are according to any one of the following rows in the table:

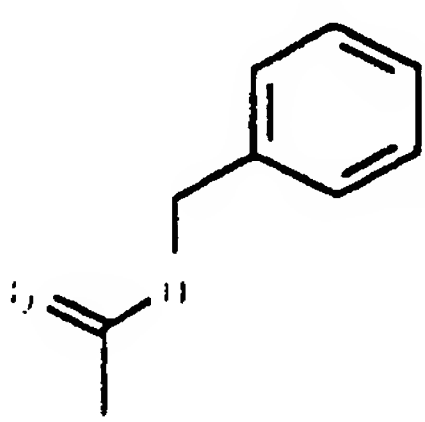
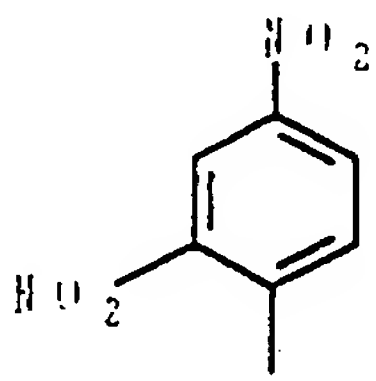
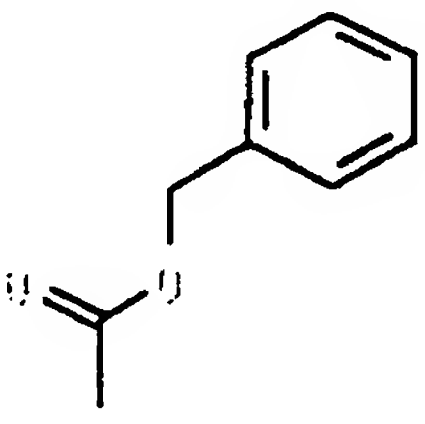
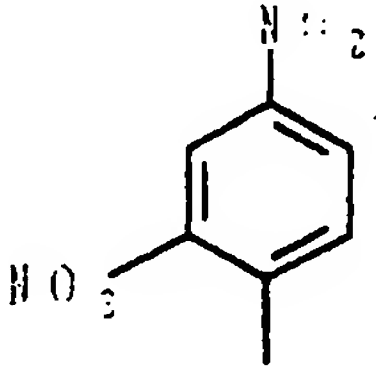
LABEL	R_2	R_1	R_4	R_3
CD4			OMe	OMe

10

CD6	CH_3CO		OH	OH
CD7			OMe	OH
CD8		$\text{CH}_2\text{-COOEt}$	OH	OH

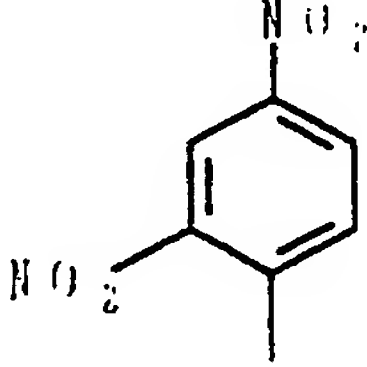
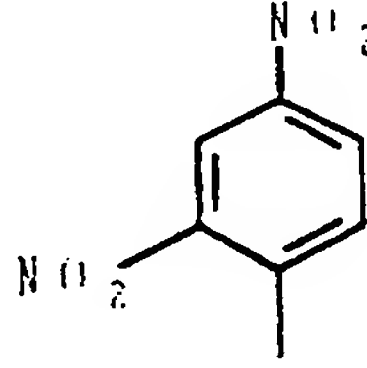
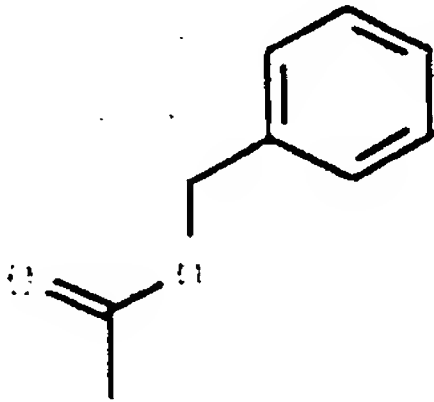
CD10			OMe	OMe
CD13	H		OH	OH
CD16	CHO		OH	OH
CD17			NH ₂	OH

21

CD19			OMe	OH
CD20			OMe	OMe
CD37	H	$ \begin{aligned} &-\text{CH}_2\text{CH}=\text{C}(\text{CH}_3) \\ &)\text{CH}_2\text{CH}_2\text{CH}=\text{C}(\text{CH}_3) \\ &\text{CH}_2\text{CH}_2\text{CH} \\ &=\text{C}(\text{CH}_3)_2 \end{aligned} $	OH	OH

SUBSTITUTE SHEET (RULE 26)

22

CD42			OMe	OH
CD43			OMe	OMe
CD46		-CH ₂ -CO ₂ Et	OMe	OMe

SUBSTITUTE SHEET (RULE 26)

23

CD48	CH ₃ CO		OMe	OH
------	--------------------	--	-----	----

2. A pharmaceutically acceptable composition for
5 delivery to the human or animal body comprising a
compound according to claim 1.
3. A composition for the treatment of parasitic
infection, which composition comprises one or
10 more of CD7, CD19, CD20, CD42, CD43 or CD44
according to claim 1.
4. A composition for the treatment of
trypanosomiasis, and in particular infection by
15 T. Brucei, which composition comprises one or
more of CD7, CD19, CD20, CD42, CD43 and CD44
according to claim 1.
5. A composition as for the treatment of
20 Leishmaniasis, and in particular infection by L.
donovani, which composition comprises one or
more of CD20, CD42, CD43, CD44 and CD46 according

SUBSTITUTE SHEET (RULE 26)

to claim 1.

6. A composition for the treatment of cancer, which
composition comprises one or more of CD4, CD6,
5 CD13 and CD37 according to claim 1.

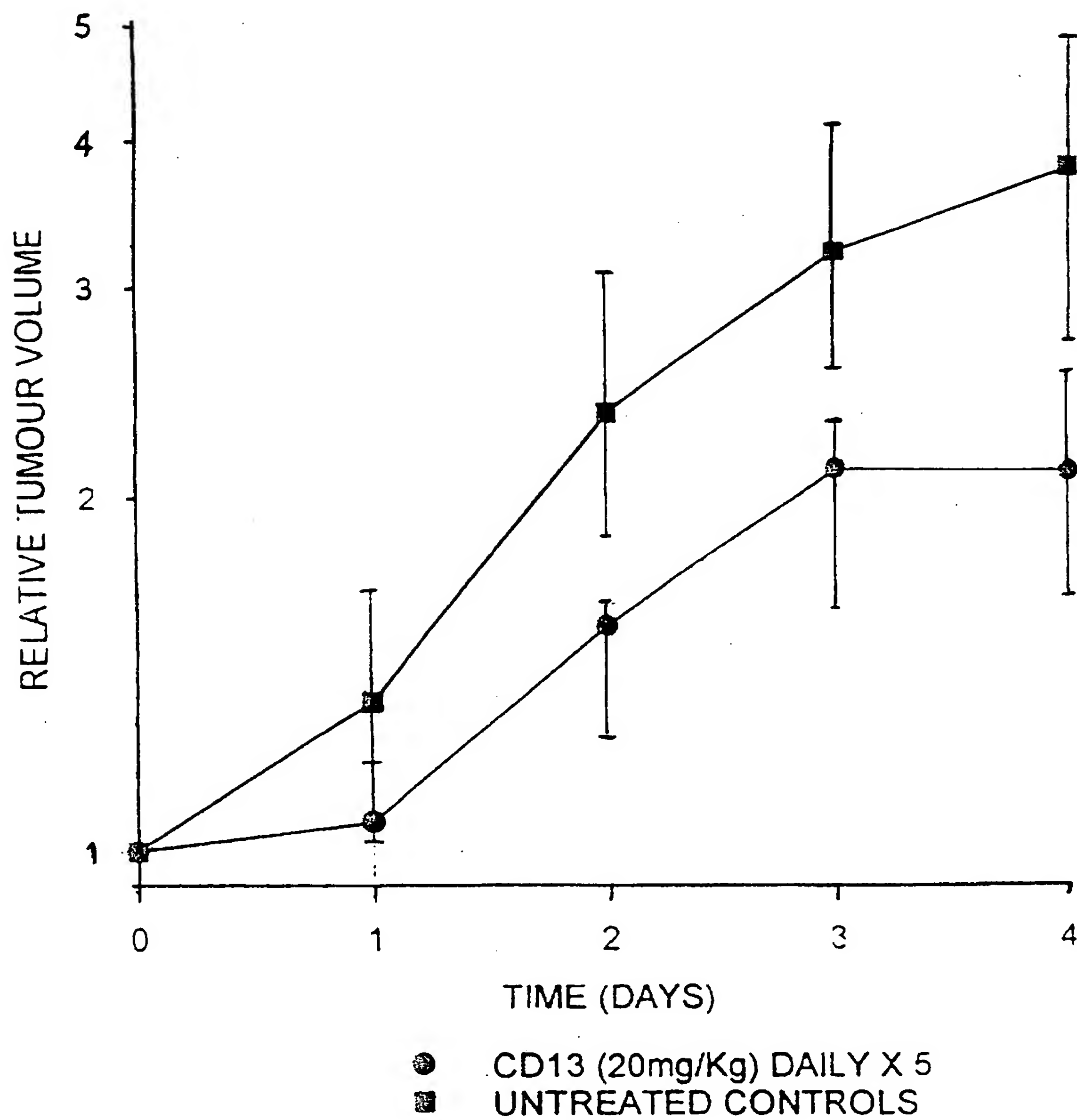
7. A method of treating a diseased human or animal
comprising administering a pharmologically
effective amount of a composition as claimed in
10 claim 2.

8. A method of treating parasitic infection of a
human or animal comprising administering a
pharmologically effective amount of a composition
15 as claimed in any of claims 3,4 and 5.

9. A method of treating cancer in a human or animal
comprising administering a pharmologically
effective amount of a composition as claimed in
20 claim 6.

1/1

MAC15A S/C TUMOURS TREATED WITH CD13 DAILY



INTERNATIONAL SEARCH REPORT

Intern. Application No

PCT/GB 97/02358

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/02 A61K38/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R.VINC ET AL.: "Studies on the Inhibition of Glyoxalase I by S-Substituted Gluthationes" J.MED.CHEM., vol. 14, no. 5, 1971, page 402-4 XP002044716 see paragraph 1; example 39; table 1 ---	1,6-9
X	A.AL-TIMARI ET AL.: "Inhibition of mammalian glyoxalase I (lactoylglutathione lyase) by N-acylated S-blocked glutathione derivatives as a probe for role of the N-site of glutathione in glyoxalase I mechanism" BIOCHIM.BIOPHYS.ACTA, vol. 86, no. 1, 1986, pages 160-8, XP002044717 see tables 12,5 --- -/-	1,6-9

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

B document member of the same patent family

Date of the actual completion of the international search

27 October 1997

Date of mailing of the international search report

13. 11. 97

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INTERNATIONAL SEARCH REPORT

Intern. Appl. No.

PCT/GB 97/02358

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A.AL-TIMARI ET AL.: "Inhibition of glutathione derivatives of bovine liver glyoxalase II (Hydroxyacylglutathione hydrolase) as a probe of the N- and S-sites for substrate binding" BIOCHIM.BIOPHYS.ACTA, vol. 870, no. 2, 1986, page 219-25 XP002044718 see example 2; table I	1,6-9
A	--- S.J.NORTON ET AL.: "Inhibitors and inhibition of mammalian glyoxalase II activity" BIOCHEM.SOC.TRNS., vol. 21, no. 2, 1993, pages 545-9, XP002044719	
A	--- NORTON S.J. ET AL.: "Glyoxalase and Glyoxalase II from Aloe Vera: Purification, Characterization and Comparision with Animal Glyoxalases" BIOCHEM.INT., vol. 22, no. 3, 1990, pages 411-18, XP002044720	
A	--- AC. ELIA ET AL.: "N,S-Bis-Fluorenylmethoxycarconylglutathione: A New, Very Potent Inhibitor of Mammalian Glyoxalase II" BIOCHIM.MOL.BIOL.INT., vol. 35, no. 4, 1995, pages 763-71, XP002044721	
A	--- WO 95 08563 A (TERRAPIN TECH INC) 30 March 1995 -----	

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Information on patent family members

Intern. Application No

PCT/GB 97/02358

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		AU 7842194 A	10-04-95
		CA 2171453 A	30-03-95
		EP 0720620 A	10-07-96
		JP 9506336 T	24-06-97
		US 5556942 A	17-09-96
